

# Time-Resolved Reflectance Spectroscopy for the Non-Destructive Detection of Inner Attributes and Defects of Fruit

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## Abstract

A review of the main results obtained by Time-resolved Reflectance Spectroscopy (TRS) and of its possible applications for the detection of inner attributes and defects in fruit is presented. Common spectroscopy techniques employ continuous wave light and measure the diffusively remitted intensity which is determined by both the absorption and the scattering properties of the sample. Light absorption is related to chemical properties, while scattering to physical structure. Quantification of absorption and scattering may be obtained by space- or time-resolved methods. TRS is a technique based on the measurement of the temporal delay and broadening experienced by a short laser pulse while traveling through a turbid medium and explores a volume of pulp at a depth of 1-2 cm. TRS has been used to detect some internal defects and disorders in the intact fruit, such as brown heart, internal browning, internal bruises, water soaked tissue, as well as internal attributes related to maturity, texture and cell wall structure. The scattering coefficient was found to be related to the translucency of tissue and was used to detect internal bruises and water soaked tissue (i.e., water core in apples and *Botrytis* decayed kiwifruits). The scattering coefficient was related to pectin composition and textural properties of apples such as firmness and mealiness. Absorption coefficient in the 600-700 nm region can detect brown heart in pears and internal browning in apples. The absorption coefficient at 670 nm is related to fruit maturity and it was used in nectarines to predict their softening time and to detect too immature fruit which are not able to ripen. Being non-destructive, TRS measurements can be repeated on the same fruit, following the development and the changes occurring with ripening or storage.

## INTRODUCTION

Optical techniques are widely studied and used for quality assessment of fruits because they are non destructive. Non destructive measurements can be applied to the entire batch of fruit before marketing without impairing its quality, while destructive measurements can only be applied to a sample.

Classical VIS/NIR spectroscopy techniques employ continuous wave light and measure the diffusively remitted intensity. In the near infrared (NIR) region, the spectrum of re-emitted light has been studied mainly to estimate the soluble solids content, acidity and texture attributes in reflectance or transmittance (Nicolai et al., 2005). In NIR reflectance spectroscopy the penetration depth is relatively small (1-5 mm) depending on the wavelength. NIR reflectance and transmittance spectra are affected by both absorption and scattering processes. VIS/NIR spectroscopy can only provide information on light

attenuation, but does not allow the non-destructive assessment of both the absorption and scattering coefficients in diffusive media. As a result, continuous wave spectroscopy requests recalibration for each new batch of samples. On the contrary, time-resolved reflectance spectroscopy (TRS) and space-resolved reflectance spectroscopy (SRS) provide a complete characterization with the simultaneous non-invasive measurement of the optical properties (absorption and scattering) of diffusive media (Cubeddu et al., 2001; Lu, 2004; Torricelli et al., 2008). Absorption and scattering contain distinct information on the medium. The absorption coefficient is determined by pigments (chlorophyll, carotenoids) and chemical constituents of the pulp (water, sugar), while the scattering coefficient is mainly due to physical structure, as the photon path can be deviated by changes in the refractive index due to the presence in the tissue of membranes, cell walls, air, vacuoles, starch granules or organelles.

In TRS, a short pulse of monochromatic light is injected into the fruit: whenever a photon strikes a scattering centre, it changes its trajectory and keeps on propagating in the tissue, until it is eventually reemitted across the boundary, or it is captured by an absorbing centre. The temporal distribution of the re-emitted photons at a distance from the injection point will be delayed, broadened, and attenuated. By an appropriate theoretical model of light penetration for the analysis of time distribution, it is possible to simultaneously measure, as a function of wavelength, the scattering coefficient  $\mu_s$  (units are typically  $\text{cm}^{-1}$ ) and the absorption coefficient  $\mu_a$  ( $\text{cm}^{-1}$ ) which indicate the scattering probability per unit length and the absorption probability per unit length, respectively. To account for non-isotropic propagation of photons the reduced scattering coefficient  $\mu'_s = (1 - g) \mu_s$  is commonly used, where  $g$  is the anisotropy factor, i.e., the mean cosine of the scattering angle. Usually, the laser light is injected into and collected from the fruit pulp by using two optical fibers placed in contact with the surface at a distance of 1-2 cm. The laser light probes a banana-shaped volume of tissue to a depth of the same order (1-2 cm), so this technique can be used to explore inner properties of fruit pulp. Typical absorption peaks in sound fruit are around 970 nm for water and 670 nm for chlorophyll. Scattering in fruit is much higher than absorption:  $10\div 25 \text{ cm}^{-1}$  vs.  $0.05\div 0.5 \text{ cm}^{-1}$  respectively (Cubeddu et al., 2002).

This paper will review the main results obtained by time-resolved reflectance spectroscopy (TRS) for non-destructive quality assessment of fruits and vegetables.

## MATERIALS AND METHODS

The two key points in designing a system for time-resolved measurements are temporal resolution (events in the range 1-10 ns) and high sensitivity (ratio of detected to injected power is about -80 dB).

Two different systems for TRS measurements of food quality have been developed at the Politecnico di Milano - Department of Physics. Both systems are based on the time-correlated single photon counting technique (TCSPC) and exploit the expertise of the research group in the biomedical field. The first one is a fully automated laboratory set-up for broadband time-resolved reflectance measurements in the spectral range 600-1000 nm. The second system is a portable prototype for time-resolved reflectance measurements at discrete wavelengths. A detailed description of the two systems can be found in Torricelli et al. (2008). In both systems, a couple of 1 mm fibers delivers light into the sample and collects the emitted photons. The fibers are positioned 1.5 cm apart, parallel to each other, normal to and in contact with the sample surface.

The temporal profile of the time-resolved reflectance curve is analysed using a solution of the Diffusion Equation for a semi-infinite homogeneous medium (Patterson et al., 1989). This allows for the simultaneous estimate of  $\mu_a$  and  $\mu'_s$ . A detailed description of the fitting procedure can be found in Cubeddu et al. (2001).

## RESULTS

### Scattering and Texture

The scattering coefficient is related to fruit structure and texture. In kiwifruit, Valero et al. (2004), using  $\mu'_s$  measured at 675 and 750 nm, obtained a model for firmness having an  $R^2$  of 0.6, which rose to 0.8 when absorption spectra were added to the model. In pears, Nicolai et al. (2008) by using TRS in the NIR region, from 875 to 1030 nm, found that firmness considerably increased with increasing  $\mu'_s$  at 900 nm, following a non-linear relationship, and this fact prevented the construction of calibration models for firmness using  $\mu'_s$  spectra. Valero et al. (2005) studied classification models based on TRS measurements at 672, 750, 818, 900 and 950 nm, in order to identify meakiness in apples. The performance of these models ranged from 47 to 100% of correctly identified mealy versus non-mealy apples. In 'Braeburn' apples after storage, the scattering coefficients at 790 and 912 nm were positively correlated to sensory meakiness and to the relative internal space volume, and negatively correlated to sensory crispness, juiciness and firmness and to percent juice (Vanoli et al., 2009b). Mealy apples had values of  $\mu'_s$  higher than  $19 \text{ cm}^{-1}$ . Similarly, in 'Jonagored' apples, Vanoli et al. (2007b) found that, after storage,  $\mu'_s$  780 was negatively correlated to firmness, percent juice and sensory crispness and positively to meakiness. In this cultivar, values of  $\mu'_s$  780 lower than  $11 \text{ cm}^{-1}$  characterised crispy, not mealy fruit, with firmness higher than 50 N and percent juice higher than 30%. After storage, scattering coefficients  $\mu'_s$  at 750 and 780 nm showed a high and positive correlation with galacturonic acid content in water soluble pectin (WSP), and a negative correlation with firmness, residue insoluble pectin (RIP) and protopectin index (PI) (Vanoli et al., 2009a) (Table 1). It seems that the hydrolysis of polysaccharides occurring during ripening (i.e., increase of WSP and decrease of RIP and PI, related also to fruit softening) could induce a decrease in the size and an increase in the number of the scattering centers, so increasing scattering values in softer fruit, according to Mie theory. The correlation between scattering and texture was found in apples after storage, but not at harvest: probably the presence of starch granules in apple fruit at harvest affects the measurements of the optical properties.

Scattering coefficient typically decreases when fruit tissue becomes translucent, like in overripe fruit, as well as with mechanical damage and fungal decay (Eccher Zerbini et al., 2002). In kiwifruit, by using a scattering coefficient at 630 nm ( $\mu'_s$  630), it was possible to distinguish unripe from ripe kiwifruit, and within the same individual fruit, after cold storage, the sound region from that affected by *Botrytis*, that was characterized by a higher translucency, corresponding to lower  $\mu'_s$  630 (Eccher Zerbini et al., 2008).

### Internal Disorders

Apples and pears, as also other fruits, under specific conditions may develop internal physiological disorders which are visible only when fruit are cut open. Among them, internal browning may show different locations of symptoms, related to different factors, such as diffuse flesh browning, radial browning (related to senescent breakdown) and brown heart (related to  $\text{CO}_2$  injury). Brown heart was detected in 'Conference' pears by an increase in the absorption coefficient  $\mu_a$  at 720 nm, which was significantly higher than that in sound tissue. Sound tissue had  $\mu_a$  at 720 nm  $\leq 0.04 \text{ cm}^{-1}$  (Eccher Zerbini et al., 2002). In 'Granny Smith' apples, the  $\mu_a$  750 increased with the development of internal browning, with healthy fruits showing the lowest values of  $\mu_a$  750 and those with brown flesh the highest (Table 2) (Vanoli et al., 2009b). The  $\mu_a$  750 was correlated to pulp colour (positively to  $a^*$ ,  $b^*$  and  $C^*$  and negatively to hue and  $L^*$ ). The  $\mu_a$  750 made possible to distinguish healthy fruit from those with brown flesh, as the former showed  $\mu_a$  750 values below  $0.030 \text{ cm}^{-1}$  and the latter  $\mu_a$  750 values above  $0.033 \text{ cm}^{-1}$ . When internal browning affected only the core region of the apple,  $\mu_a$  750 ranged from 0.030 to  $0.035 \text{ cm}^{-1}$ .

Watercore develops in some apple cultivars at harvest, often on the sunny side of the fruit; the affected areas look glassy and translucent due to the presence of water in the intercellular spaces, instead of air. The water-soaked tissue is usually located around the

vascular bundles or the core area. In 'Fuji' apples, healthy pulp was characterized by significantly lower  $\mu_a$  790 and higher  $\mu'_s$  790 than zones affected by watercore (Table 2) (Vanoli et al., 2009b).

### **Fruit Maturity**

Chlorophyll absorbs light with a peak at 672 nm. The use of absorption at a wavelength near the chlorophyll peak (between 630 and 690 nm) as a maturity index (high  $\mu_a$  = less mature fruit, low  $\mu_a$  = more mature fruit) has been checked in different fruit species, by comparing  $\mu_a$  measured at harvest to fruit quality after storage and shelf life. 'Jonagored' apples classified as more mature by TRS had less titratable acidity at harvest and more soluble solids after storage; at sensory analyses these fruits were significantly sweeter, more aromatic and pleasant. However, flesh firmness in apples was not related to maturity class (Vanoli et al., 2005). Similar results were obtained with 'Abate Fétel' pears (Eccher Zerbini et al., 2005). In 'Spring Bright' nectarines with lower  $\mu_a$  670 at harvest, during shelf life the soluble solids content and percent blush was higher, firmness and acidity lower than in fruit with high  $\mu_a$  670. As regards sensory attributes, more mature 'Spring Bright' and 'Ambra' nectarines were perceived significantly less firm and more juicy, sweet, pulpy and aromatic and were more appreciated by the assessors (Eccher Zerbini et al., 2003; Vanoli et al., 2008). Nectarines selected by TRS had also different quality as regards sugar, acid and volatile compositions. Total sugars, percent sucrose, malic and quinic acid increased while percent fructose, sorbitol, total acids and especially citric acid decreased with decreasing  $\mu_a$  670 (Jacob et al., 2006). In 'Ambra' nectarines the odour pattern changed with  $\mu_a$  and with shelf life at 20°C: the "green" note due to aldehydes was more present in fruit with higher  $\mu_a$  670, while the "fruity, sweet, peach-like" odour associated to lactones was preeminent in the more mature ones (low  $\mu_a$  670) (Vanoli et al., 2008).

The trend of  $\mu_a$  during maturation and ripening of nectarine fruit has been studied by measuring  $\mu_a$  670 on the same fruit during a storage period of five days at two temperatures (10 and 20°C). It was found that  $\mu_a$  670 decayed with storage time following a logistic curve. This decay occurs in all fruit with the same rate during ripening, although shifted in time from fruit to fruit according to maturity (Tijskens et al., 2006). In this way it was assumed that variability in the maturity of nectarines at the time of harvest is largely due to the individual age of the fruit at the tree. By using the non-linear mixed effects regression analysis, it was possible to estimate for each fruit the biological time-shift factor (BSF), which indicates the time required to reach a reference stage of maturity, such as, e.g., the midpoint of the logistic curve of  $\mu_a$  decay. By applying the individual BSF, the results indicated that the mechanism of  $\mu_a$  670 decay is the same or very similar in fruits both at the plant and after harvest, so that  $\mu_a$  can really be used as an index of fruit age and hence maturity. The biological variance that exists in measured values of  $\mu_a$  is transformed by this method into variation in maturity, expressed as time to ripen until the midpoint of the logistic curve (Tijskens et al., 2006). The BSF contains all the information on the stage of maturity at harvest. Its distribution is normal, which greatly enhances the usability of the factor in determining the maturity at harvest and the biological variance in batches of peaches and nectarines.

The most interesting result obtained so far from the use of  $\mu_a$  670 for the evaluation of maturity, is its predictive power as regards softening of nectarine fruit (Eccher Zerbini et al., 2006; Tijskens et al., 2007). Softening occurred earlier in more mature fruit (low  $\mu_a$  670 at harvest) and later in less mature fruit (high  $\mu_a$  670 at harvest), with the same sigmoid pattern in time (Eccher Zerbini et al., 2006). At the same time, in fruit with lower  $\mu_a$  670 at harvest, ethylene production was accelerated reaching earlier the climacteric (Vanoli et al., 2007a). For firmness decay, a logistic model was developed by integrating the biological time-shift derived from the measurement of  $\mu_a$  with the kinetics of fruit softening process (Fig. 1) (Tijskens et al., 2007). From the developed model and the analyses of six data sets in 4 years, it can be concluded that the value of  $\mu_a$  at harvest is directly related to the maturity of fruit at the moment of harvest and to its ripening

potential, so that the future firmness decay of individual fruit can be predicted from the measurement of  $\mu_a$  670 at harvest (Eccher Zerbini et al., 2009). Size of the fruit had no significant effect either on the fruit softening or on the  $\mu_a$  decay during storage. The softening kinetics however depend on the length of the cold storage (Rizzolo et al., 2009).

## DISCUSSION

TRS showed interesting results in relation to fruit texture and to the detection of disorders. However, if the disorder is at a depth higher than 2 cm, it cannot be detected with the setup used in this experiment, as the penetration depth of the detected light is of the order of the distance between the two optical fibres. Furthermore, it is necessary to determine the TRS threshold values specific to the cultivars for each disorder, and, in order to make certain the detection of the defect, the number of measurement points has to be suited to the localization and distribution of the affected tissue.

The application of TRS to evaluation of maturity and to prediction of softening, combining non-invasive measuring techniques, process oriented modeling, application of biological shift factor theory and non-linear regression analysis, may have considerable commercial implications for harvesting nectarines, peaches and most probably other stone fruits. Ongoing research with mangoes is also showing promising results.

For on-line grading of fruits, some aspects still need to be studied (non-contact measurement, number of points, etc.), but a great deal of potential applications can be envisaged for this technique when a simpler instrumentation will be available. The time required for one TRS measurement is typically 1 s with the portable prototype, but the technique could be adapted for on-line measurement, reducing acquisition time to 10 ms without loss of accuracy. While laser sources, photo-detectors and electronics for TCSPC can be separately bought from different dealers, to our knowledge no commercial systems for TRS measurements are nowadays available on the European and US market. The presented systems are far to be the ultimate setup for TRS due to limitations in laser source power, temporal resolution and number of wavelengths. Research to investigate innovative schemes has therefore been started at Politecnico of Milan. Next generation TRS setup will possibly exploit broadband and narrow light source and detection tools with high temporal resolution and extended spectral sensitivity. Work is in progress to develop a new setup for food quality assessment using new and compact components, already tested in medical applications, and innovative tools for both light delivery and light detection which will further enhance TRS performances in the field of post-harvest and quality sensing.

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## **Tables**

Table 1. Correlation coefficients of scattering coefficients ( $\mu'_s$ ) at 750 and 780 nm measured after storage with firmness, galacturonic acid content in water soluble and residual insoluble pectin fractions, and protopectin index (from Vanoli et al., 2009a).

	$\mu'_s$ 750	$\mu'_s$ 780
Firmness	-0.81***	-0.78***
Water soluble pectin	0.77***	0.75***
Residue insoluble pectin	-0.66***	-0.62**
Protopectin index	-0.84***	-0.81***

Table 2. Mean values of selected optical parameters in Braeburn and Fuji apples affected by internal disorders (from Vanoli et al., 2009b).

Braeburn apples	Non mealy	mealy	Fuji apples	healthy	watercore
$\mu'_s$ 912 (cm <sup>-1</sup> )	16.26	19.47***	$\mu'_a$ 790 (cm <sup>-1</sup> )	0.049	0.059***
$\mu'_s$ 790 (cm <sup>-1</sup> )	16.41	20.13***		$\mu'_s$ 790 (cm <sup>-1</sup> )	8.99

## Figures

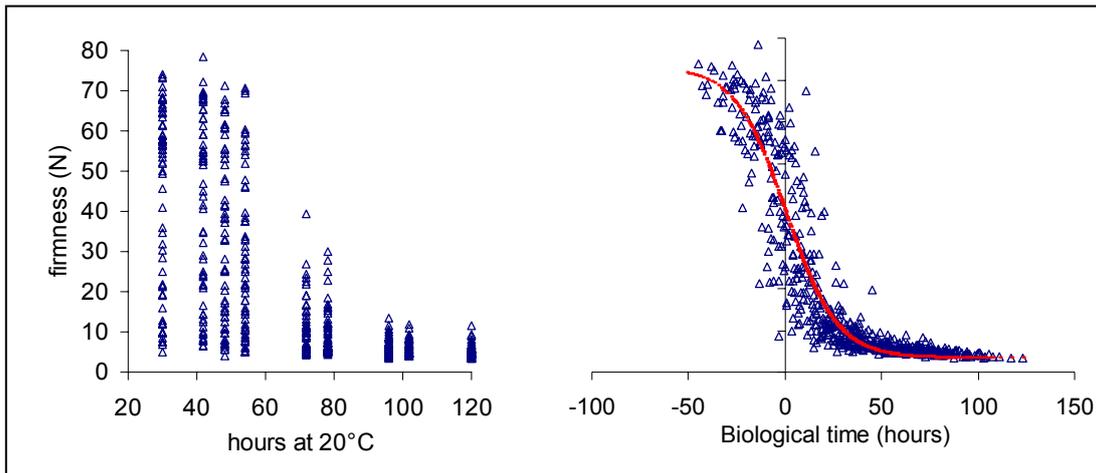


Fig. 1. Firmness of 'Spring Bright' nectarines measured during shelf-life at 20°C (left) and after correction for biological shift factor of individual fruit, which is based on the  $\mu_{a670}$  measured at harvest (right). The solid line represents the logistic softening model.